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Berberis lycium: the newly discovered aecial host of *Puccinia striiformoides*

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Abstract—*Berberis lycium* is reported for the first time as the alternate (aecial) host for *Puccinia striiformoides* from collections in Pakistan. This finding is confirmed by phylogenetic analyses of internal transcribed spacer (ITS) rDNA sequence data, which place our sequences of aecial *P. striiformoides* on *B. lycium* in a well-supported clade with GenBank sequences from uredinial *P. striiformoides* on *Dactylis glomerata*. Aecia of *Puccinia graminis*, *P. graminis* f.sp. *tritici*, and *P. brachypodii* that were also collected on *B. lycium* from Pakistan provide additional evidence of multiple rust species occurring on secondary host plant species.

Key words—*Berberidaceae*, *Pucciniales*, rust fungi, *Uredinales*

Introduction

About 400 species of rust fungi (*Pucciniales*) in 50 genera and five form genera on 350 plant species have been reported from Pakistan (Ahmad & al. 1997, Afshan & Khalid 2013). These include c. 80 species of rust fungi on ~93 host plants in the *Poaceae* recorded from Pakistan (Afshan & Khalid 2013).

Puccinia Pers. contains numerous species of heteroecious rust fungi with a life cycle that involves different spore stages on unrelated alternate hosts: uredinial, telial, and basidial stages on a primary host and pycnial (spermatial)

and aecial stages on a secondary host. *Berberis* spp. (barberries) have been reported as secondary hosts for several *Puccinia* species: *P. brachypodii* G.H. Otth, *P. brachypodii-phoenicoidis* Guyot & Malençon, *P. graminis* Pers., *P. montanensis* Ellis, *P. pseudostriiformis*, *P. pygmaea* Erikss., and *P. striiformis* Westend. (Berlin & al. 2013). Specifically, *B. lycium* has been reported as the alternate host for *P. brachypodii, P. graminis*, and *P. graminis* f.sp. *tritici* Erikss. & Henning (Staletić & al. 2004, Gautam & Avasthi 2016). Recently, *Berberis* sp. was revealed as an alternate host of *P. striiformis* through molecular barcoding of the internal transcribed spacer region (ITS) (Jin & al. 2010).

Berberis lycium is a medium-sized evergreen shrub widely distributed in northern Pakistan (Sood & al. 2012). It is used as food and in folk medicines. Many important phytochemicals have been identified in this species, which possesses antidiabetic, antihyperlipidemic, hepatoprotective, antibacterial, antifungal, anticoccidial, pesticidal, antimutagenic, and wound healing properties (Sher & Al- Yemeni 2011, Shabbir & al. 2012).

Phylogenetic analyses of the ITS region in combination with the large subunit (LSU) have been used to identify c. 200 *Puccinia* species (Jin & al. 2010, McTaggart & al. 2016a, Marin-Felix & al. 2017, Ali & al. 2017). However, almost no effort has been made to use phylogenetic methods to establish the linkages between aecial and uredinial/telial specimens with previously unknown heteroecious life-cycles (e.g., Padamsee & McKenzie 2017).

In the past, two varieties were known for yellow or stripe rust, *Puccinia striiformis*: *P. striiformis* var. *striiformis* and *P. striiformis* var. *dactylidis* (Cummins 1971). However, Abbasi & al. (2005) resolved the *P. striiformis* species complex into three distinct species using morphological and molecular approaches; their ITS sequence data resolved the three species as *P. striiformis*, *P. pseudostriiformis*, and *P. striiformoides*.

Puccinia striiformoides has been reported on *Poa pratensis* L. from Czech Republic and on *Dactylis glomerata* L. from Canada, Chile, China, Czech Republic, Hawaii, Iran, New Zealand, United Kingdom, and USA (Farr & Rossman 2017). *P. striiformoides* is also reported from Pakistan on *Lolium perenne* L. with uredinial and telial stages (Afshan & al. 2007). Here we present the first record of the aecial stage of *P. striiformoides* and the first record of *Berberis lycium* as an alternate host of this rust species.

Materials & methods

Rust infected *Berberis lycium* was collected during July–August 2014 from areas where barberry commonly grows at altitudes 1800–2400 m near the Punjab/ Khyber Pakhtunkhwa border, Pakistan. Infected plants were found in regions with a

temperature range of 20–30°C during the day and 16–21°C at night, with an average humidity of 40–50%.

Berberis lycium leaves infected with aecia were collected and kept in newspaper and paper envelopes for preservation. After air-drying for three days, aecia were excised from a single local lesion and preserved at –80ºC for later DNA extraction. *Triticum aestivum* plants infected with *Puccinia* (uredinia) were collected from Khyber Pakhtunkhwa, Punjab, and Sind during March 2014. The urediniospore samples of leaf rust were purified and multiplied under glass house conditions following Bariana & McIntosh (1994). The urediniospore isolates were preserved at –80°C for DNA extraction.

DNA was extracted from dried aecia and uredinia using a modified cetyltrimethylammonium bromide (CTAB) method (Ali & al. 2016). The extracted DNA samples were quantified in a NanoDrop™ 3300 Fluorospectrometer and 30 ng/µl was used for PCR amplification of ITS and 18S rDNA regions. The ITS region was amplified using ITS5/ITS4 primers (Justesen & al. 2003) and the SSU region using universal primers FG-F/FG-R (Einsele & al. 1997). The ITS and SSU PCR products of aecial and uredinial isolates were purified using Thermo Scientific™ GeneJET PCR purification kit according to the manufacturer's instructions and commercially sequenced by Macrogen in Korea. Raw sequence data were edited using BioEdit (Hall 1999), and sequences were deposited in GenBank.

The ITS sequences of aecial and uredinial isolates were BLASTed against the GenBank database (www.ncbi.nlm.nih.gov). A total of 34 ITS sequences for *Puccinia* species previously deposited in NCBI and the sequences generated in this study was used for the phylogenetic analysis. ITS sequences of aecial and uredinial isolates were aligned using Muscle E multiple alignment tool within MEGA v. 7.0 (Kumar & al. 2016). Phylogenetic analysis of the ITS sequences was performed using maximum likelihood (ML) method based on General Time Reversible Model (GTRMGAMMA; Nei & Kumar 2000). The percentage of trees in which the associated taxa cluster together is shown next to the branches (Fig. 1). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. All positions containing gaps and missing data were eliminated. There were a total of 248 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7.0 (Kumar & al. 2016). *Uromyces dactylidis* G.H. Otth was used as outgroup in phylogenetic analyses (Liu & Hambleton 2010).

Phylogenetic results

NCBI BLASTn analysis revealed that our ITS sequences matched closely with sequences of *P. striiformoides* in GenBank (Fig. 1). PAK-7 showed 99% identity with sequences from Canada (HM057110; 423/429 bp), China (HM057137; 337/340 bp), and South Africa (KP893702; 418/424); PAK-14 also showed 99% identity with sequences from Canada (HM057110;

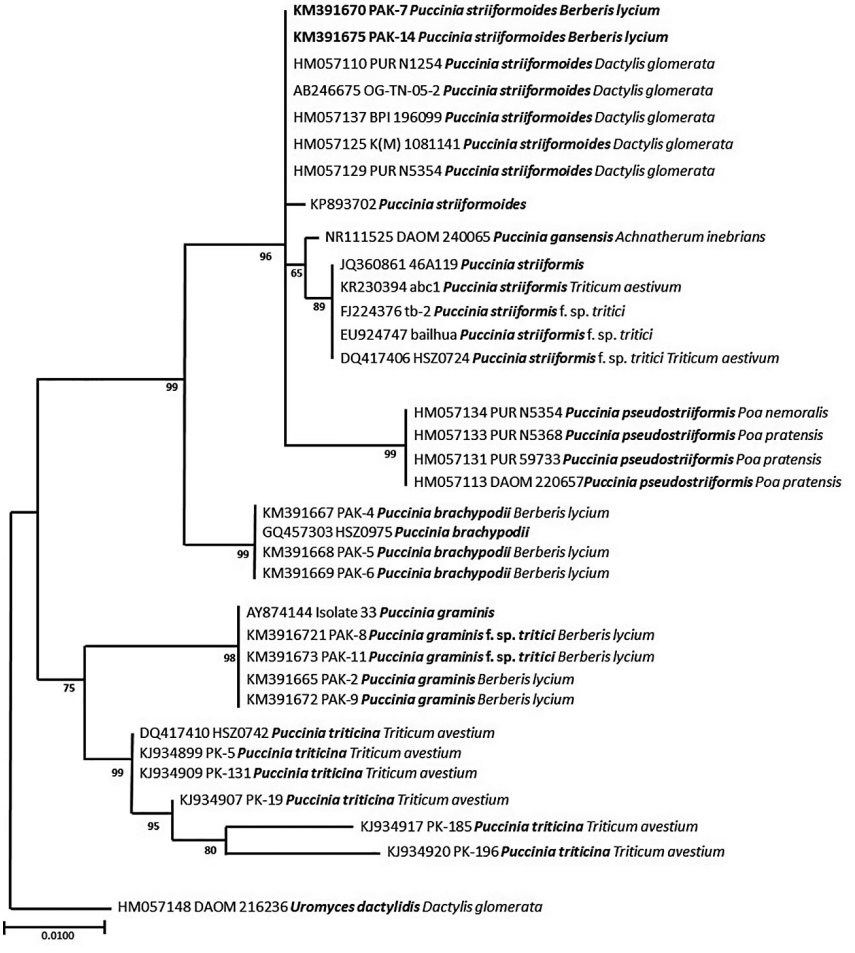
KM391670 PAK-7 Puccinia striiformoides Berberis lycium
KM391675 PAK-14 Puccinia striiformoides Berberis lycium
HM057110 PUR N1254 Puccinia striiformoides Dactylis glomerata
AB246675 OG-TN-05-2 Puccinia striiformoides Dactylis glomerata
HM057137 BPI 196099 Puccinia striiformoides Dactylis glomerata
HM057125 K(M) 1081141 Puccinia striiformoides Dactylis glomerata
HM057129 PUR N5354 Puccinia striiformoides Dactylis glomerata
KP893702 Puccinia striiformoides
NR111525 DAOM 240065 Puccinia gansensis Achnatherum inebrians
JQ360861 46A119 Puccinia striiformis
KR230394 abc1 Puccinia striiformis Triticum aestivum
FJ224376 tb-2 Puccinia striiformis f. sp. tritici
EU924747 bailhua Puccinia striiformis f. sp. tritici
DQ417406 HSZ0724 Puccinia striiformis f. sp. tritici Triticum aestivum
HM057134 PUR N5354 Puccinia pseudostriiformis Poa nemoralis
HM057133 PUR N5368 Puccinia pseudostriiformis Poa pratensis
HM057131 PUR 59733 Puccinia pseudostriiformis Poa pratensis
HM057113 DAOM 220657 Puccinia pseudostriiformis Poa pratensis
KM391667 PAK-4 Puccinia brachypodii Berberis lycium
GQ457303 HSZ0975 Puccinia brachypodii
KM391668 PAK-5 Puccinia brachypodii Berberis lycium
KM391669 PAK-6 Puccinia brachypodii Berberis lycium
AY874144 Isolate 33 Puccinia graminis
KM3916721 PAK-8 Puccinia graminis f. sp. tritici Berberis lycium
KM391673 PAK-11 Puccinia graminis f. sp. tritici Berberis lycium
KM391665 PAK-2 Puccinia graminis Berberis lycium
KM391672 PAK-9 Puccinia graminis Berberis lycium
DQ417410 HSZ0742 Puccinia triticina Triticum avestium
KJ934899 PK-5 Puccinia triticina Triticum avestium
KJ934909 PK-131 Puccinia triticina Triticum avestium
KJ934907 PK-19 Puccinia triticina Triticum avestium
KJ934917 PK-185 Puccinia triticina Triticum avestium
KJ934920 PK-196 Puccinia triticina Triticum avestium
HM057148 DAOM 216236 Uromyces dactylidis Dactylis glomerata
96
65
89
99
99
99
98
75
99
95
80
0.0100

Fig. 1: A phylogram from maximum likelihood (ML) analyses based on ITS sequence data. The numbers on the branches are bootstrap values from ML analyses. Taxa in the phylogram are labeled as "accession number, strain or isolate, rust species name and host name". The ITS sequences generated in this study are represented by PAK and PK numbers. *Uromyces dactylidis* was chosen as outgroup. Bootstrap values >65 are shown at the nodes.

423/429 bp), Japan (AB246675; 423/429 bp), and South Africa (KP893702; 418/424 bp). These BLAST results had highest identity to sequences such as HM057137 and HM057110 of *P. striiformoides* made by Liu & Hambleton (2010).

Taxonomy

Puccinia striiformoides M. Abbasi, Hedjar. & M. Scholler, Rostaniha 5: 75. 2005 ["2004"].4) Plate 1

= *Puccinia striiformis* var. *dactylidis* Manners; Trans. Brit. Mycol. Soc. 43: 65. 1960.

= "*Puccinia striiformis* f.sp. *dactylidis*" H. Tollenaar, Phytopathology 57: 418. 1967.

Pycnia (Spermogonia) adaxial, 0.2–0.5 mm in diam., aggregated in small clusters; while Pycniospores are present, spots are yellowish to pinkish and surrounded by yellowish ring; after pycniospores have oozed out, spots become variously colored but usually pink to reddish surrounded by greenish ring. Aecia abaxial, yellowish, abundant, cylindrical, many individual aecia are aggregated into a single aecial sorus, aecidioid type with peridium, individual aecium raised; Aeciospores with yellowish contents, angular, elongated, subgloboid or ellipsoid, verrucose, wall thickened, hyaline, 19–26 × 16–22 µm.

Material Examined —

Puccinia striiformoides aecia on *Berberis lycium* Royle: **PAKISTAN**, **Punjab**, **Murree**, Sunny Bank, altitude 2220 m as1, July 2014, leg. Y. Sohail PAK-14 (ISL-129547; GenBank KM391675, KP056210); **Khyber Pakhtunkhwa**, **Ayubia**, altitude 2400 m as1, July 2014, leg. Y. Sohail PAK-7 (ISL-129547; GenBank KM391670, KP056206).

Puccinia brachypodii aecia on *B. lycium*: **PAKISTAN**, **Punjab**, **Murree**, Aliyot, altitude 2200 m as1, July 2014, leg. Y. Sohail PAK-4 (ISL-129548; GenBank KM391667, KP056204); **Barian**, altitude 1798 m as1, August 2014, leg. Y. Sohail PAK-5,-6 (ISL-129548; GenBank KM391668, KM391669, KP056205).

Puccinia graminis aecia on *B. lycium*: **PAKISTAN**, **Punjab**, **Murree**, Mall road, altitude 2291 m as1, August 2014, leg. Y. Sohail PAK-2 (ISL-129549; GenBank KM391665); **Patriata**, Surasi, altitude 2285 m as1, August 2014, leg. Y. Sohail PAK-9 (ISL-129549; GenBank KM391672).

Puccinia graminis f.sp. *tritici* aecia on *B. lycium*: **PAKISTAN**, **Punjab**, **Patriata**, altitude 2285 m as1, August 2014, leg. Y. Sohail PAK-8 (ISL-129550; GenBank KM391671, KP056207); **Ghora Gali**, altitude 1900 m as1, August 2014, leg. Y. Sohail PAK-1 (ISL-129550; GenBank KM391673, KP056208).

Puccinia triticina on *T. aestivum*: **PAKISTAN**, **Punjab**, **Okara**, Chak 34 GD, altitude 105 m as1, March 2014, leg. Y. Sohail PK-19 (ISL-129551; GenBank KJ934907, KP056212); **Khyber Pakhtunkhwa**, **Nowshara**, altitude 404 m as1, March 2014, leg. Y. Sohail PK-131 (ISL-129551; GenBank KJ934909, KP056213); **Sind**, **Matiari**, Bypass, altitude 31 m as1, March 2014, leg. Y. Sohail PK-5 (ISL-129551; GenBank KJ934899, KP056211); **Tando Allahyar**, altitude 25 m as1, March 2014, leg. Y. Sohail PK-185 (ISL-129551; GenBank KJ934917, KP056214); altitude 25 m as1, March 2014, leg. Y. Sohail PK-196 (ISL-129551; GenBank KJ934920, KP056215).

Discussion

The present study established the aecial host of *P. striiformoides* as *Berberis lycium* using a molecular barcoding approach with the ITS region of rDNA.

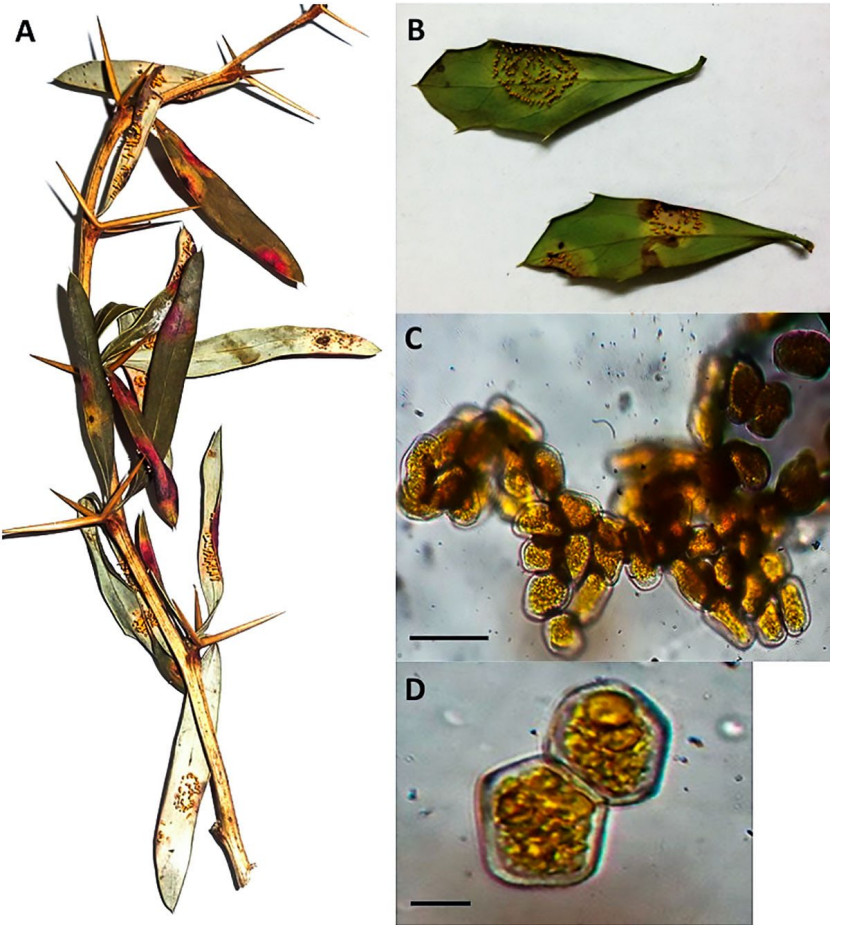

Plate 1. *Puccinia striiformoides*. A. Aecial sori on abaxial surface of leaves of *Berberis lycium*, and old pycnial spots on the adaxial surface of leaves. B. Aecial pustules. C, D. Aeciospores. Scale bars: C = 10 µm; D = 5 µm.

These findings underscore that many aecial and telial stages of rust fungi are not yet linked, a continuing challenge due to the occurrence of aecia representing many different species on one host.

Species of *Berberis* are known as alternate hosts or aecial hosts for multiple *Puccinia* species including rusts on gramineous plants, especially wheat (*T. aestivum*), throughout the world (Cummins & Greene 1966, Urban 1967,

Jin & al. 2010). Previously, barberry was associated primarily with the aecial stage of wheat stem rust (*P. graminis* f.sp. *tritici*). Various *Berberis* species have now been identified as alternate hosts of *P. striiformis* (Zhao & al. 2013), the causal agent of wheat stripe rust. However, no previous study has assessed the possible role of *B. lycium* as an alternate host for other rusts.

BLASTn and phylogenetic analyses identified our aecial isolates from one host species as representing four rust species: *P. brachypodii*, *P. graminis*, *P. graminis* f.sp. *tritici*, and *P. striiformoides*. Our phylogenetic analysis clustered two of our isolates PAK-7 and PAK-14 with uredinial sequences of *P. striiformoides* from GenBank, which constituted a strongly supported monophyletic group. Aecial isolates PAK-7 and PAK-14 (collected from *B. lycium*, Fig. 1) represent the first aecial record of *P. striiformoides*. Previously Afshan & al. (2007) described the uredinial and telial stages of *P. striiformoides* on *Lolium perenne* in the Murree region, which clearly suggests that the pathogen exists in Murree on both primary and secondary hosts. The occurrence of *P. brachypodii*, *P. graminis*, *P. graminis* f.sp. *tritici*, and *P. striiformoides* on *B. lycium* shows the pathogen pressure on the natural stands of *B. lycium* and source of inoculum for the primary hosts in Pakistan. It is highly likely that their aecial stage has remained on barberry while the uredinial/telial stages have shifted hosts. McTaggart & al. (2016b) have recently linked such a host shift or subsequent jumps with shaping the diversity of rust fungi, which suggests further collection and investigation is needed, especially of primary hosts, to identify the diversity of *Puccinia* species in Pakistan and elsewhere in the world.

Furthermore, the investigation and establishment of unknown aecial stage with other rust spore stages is important. Aime & al. (2018), recently concluded that the aecial stage plays a role in the diversification of rust species with their hosts, which means the availability of aecial stages and related hosts will improve the systematics of rust fungi.

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